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Crystal Structure of Calcineurin-Cyclophilin-Cyclosporin Shows Common but Distinct Recognition of Immunophilin-Drug Complexes

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Beamline: X12C

Introduction: Calcineurin (CN) is a serine/threonine protein phosphatase and a common receptor for cyclophilin A-cyclosporin A (CyPA-CsA) and FKBP-FK506 [1,2]. The binding of CyPA-CsA or FKBP-FK506 inhibits the CN dephosphorylation activity on the transcription factor NFAT, thus blocking T-cell activation [3].

Methods: Human CN was expressed in *E coli* and purified using a previously described procedure with slight modifications [4]. The crystals of CyPA-CsA-CN were grown by hanging drop. The trigonal crystals have the space group $P3_221$ with cell dimensions of a = b = 135.0 and c = 121.0 Å. The tetragonal crystals have the space group $P4_12_12$ with cell dimensions of a = b = 108.7 and c = 316.6 Å. The diffraction data were collected on beamlines of X12C of National Synchrotron Light Source and BioCars 14C of Advanced Photon Source and processed with the HKL software. The CyPA-CsA-CN structure was solved by molecular replacement and refined to R-factor/R-free of 0.26/0.32 at 2.8 Å resolution.

Results: The CyPA-CsA-CN structure showed that CyPA-CsA binds to the common composite surface of CN and share major recognition elements with FKBP-FK506 [5, 6]. Tyr159, Phe160, Leu312, Val314, Asn345, Trp352, Pro355 and Phe356 of CNA and Glu47, Gln50, Met118, Val119 and Asn122 of CNB are commonly recognized by both CyPA-CsA and FKBP-FK506 via hydrogen bonds or van der Waals' interactions. On the other hand, a significant number of CN residues interacting with CyPA-CsA differ from those with FKBP-FK506. Among the 25 CN residues involved in hydrogen bond or hydrophobic interaction, five are uniquely for CyPA-CsA or FKBP-FK506. In addition, the patterns of recognition are dramatically different. Of the nine hydrogen bonds between CyPA-CsA and CN, only four are common to FKBP-FK506. Specially notable is that Tyr341 forms hydrogen bond with CsA, but van der Waals interaction with Arg42 of FKBP. This recognition diversity of the composite surface may imply its capacity for binding of a variety of protein substrates.

Conclusion: Calcineurin has relatively narrow substrate specificity in comparison to other protein phosphotases. Our study shows that the structurally distinct complexes of CyPA-CsA and FKBP-FK506 share their binding sites, suggesting that the CNA-CNB composite surface may serve as a general substrate recognition site, defining substrate specificity of CN.

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References:

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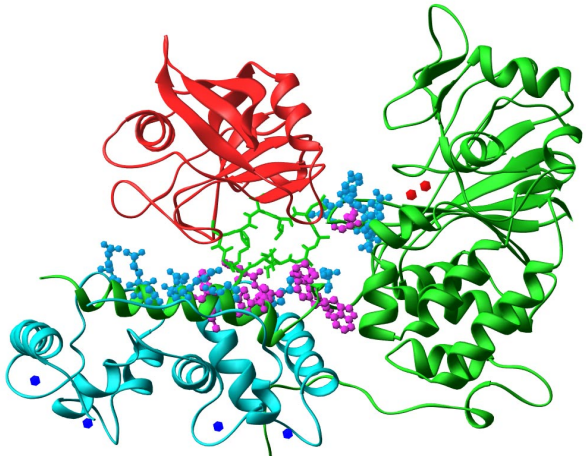

Fig. 1. Ribbon presentation of CyPA-CsA-CN. Color codes are: CNA, green; CNB, cyan; CsA, green; CyPA, Zn^{2+} and Fe^{3+}, red; and calcium, blue. The pink balls are CN residues involved in binding of CsA while cyan balls are for the CyPA binding.